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Background and Methods: Steroid-refractory acute GVHD (SR-GVHD) remains a significant complication of allogeneic hematopoietic cell transplantation (HCT). Prochymal® (Mesenchymal Stem Cells, MSC, derived from unrelated volunteer adult donors) was evaluated in addition to standard of care, including institutionally selected second line treatment, in a randomized (2:1) trial in patients with SR-GVHD (Protocol 280). Patients received 8 infusions of 2 $\times$ 10^6 MSC/kg over 4 weeks (or volume equivalent for placebo), with an additional 4 infusions administered weekly after day 28 in patients who had a partial response, defined as improvement in at least one organ without progression in others. The primary endpoint was durable complete response (DCR) for $\geq$ 28 days. Additional prospectively defined outcomes included responses in patients by organ involvement. P-values were based on Chi-square tests, unless otherwise specified.

Patients and Results: 244 patients with SR-GVHD (skin involvement n = 144, GI involvement n = 179, liver involvement n = 61) were enrolled and treated on study: Prochymal (n = 163), placebo (n = 81). There were no significant differences in age, pre-transplant conditioning, graft source, HLA-matching or second-line therapy between treatment arms. For the Prochymal and placebo arms, respectively, the grades of GVHD at entry were B (22% vs. 26%), C (51% vs. 58%), and D (27% vs. 16%). The respective DCR rates were 35% vs. 30% (p = 0.3) in the ITT population and 40% vs. 28% (p = 0.08) in the per protocol population. Results for secondary endpoints are shown in the table. Patients with GvHD affecting all 3 organs had overall complete or partial responses rate of 63% vs. 0% (n = 22, $p < 0.05$, Fisher's exact test) at day 28. Patients treated with Prochymal had less progression of liver GVHD at weeks 2 and 4 respectively (32% vs 59%, p = 0.05; and 37% vs. 65%, p = 0.05). The incidence of infections was not different between arms. Incidence rates were 9% vs. 8% for recurrent malignancy, 1.8% vs. 2.5% for infusional toxicity, and 0.6% vs. 4.6% for discontinuation due to an AE in Prochymal versus Placebo, respectively.

Conclusion: GVHD with liver or gut involvement is a life-threatening complication of HCT. Our results suggest that the addition of Prochymal produced significant improvement without additive toxicity in patients with SR-GVHD involving visceral organs.

Overall Complete or Partial Response Rates in Patients According to Organ Involvement

	Day 100 Response Rate				
Organ	Prochymal	Placebo	Odds Ratio	95% CI	P Value
Overall	82%	73%	1.7	0.9-3.1	0.12
Skin	78%	77%	1.1	0.5-2.4	0.9
Liver	76%	47%	3.6	1.1-11.2	<0.05
Gut	82%	68%	2.2	1.1-4.4	<0.05

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CRUCIAL ROLE FOR CROSS-PRESENTATION IN THE INDUCTION OF GVHD BY T CELLS DIRECTED AGAINST A SINGLE IMMUNODOMINANT MINOR HISTOCOMPATIBILITY ANTIGEN DESPITE LACK OF EPITOPE SPREADING

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The current paradigm indicates that epitope speading is critical for GVHD after MHC matched minor antigen (miHA) mismatched BMT. Because clinical data suggest that sex mismatched antigens are relevant miHAs for GVH responses, we directly tested relevance of epitope spreading by inducing GVHD in the presence of only a single immuno-dominant Y antigen disparity. We utilized female (F)→male(M) MHC matched BMT with anti-H-Y monospecific, H-2(b)-restricted T cell receptor transgenic (TCR Tg) MataHari $CD8^+$ T cells. B6 [M and F] animals were lethally irradiated and transplanted with TCD BM and 1×10^6 splenic $CD8^+$ T cells from ♀ MataHari donors. None of the syngeneic female B6 animals developed GVHD and all of them survived. By contrast, all of the allogeneic male animals that received MataHari T cells showed severe clinical GVHD and died after BMT ($P < 0.0001$, see Table). GVHD specific death was confirmed by target organ (GI tract and liver) histopathology on day +7 after BMT ($P < 0.005$). To determine if the precursor frequency alloreactive donor T cells is critical, we reduced the dose of the MataHari T cells by ten fold (1×10^5) and mixed them with syngeneic T cells (1:9 ratio) from male B6 donors. Significant GVHD mortality was observed in the MataHari group (33% vs. 0%, $P < 0.01$) demonstrating that the presence of sufficient precursor T cells against a single immuno-dominat miHA can induce significant GVHD even in the absence of epitope spreading. GVHD mortality was also observed when two other anti-H-Y specific TCR Tg $CD4^+$ (Marilyn and Rachel) were utilized as donor T cells ruling out any $CD8^+$ T cell only or strain dependent artifacts. Mechanistic studies were performed to determine whether direct and/or cross-presentation (on host or donor hematopoietic derived APCs) of the immunodominant miHA is critical for GVHD. [F → M], [II-/-→M] and [M → F] bone marrow chimeras generated, lethally re-irradiated and injected with TCD-BM and Marilyn $CD4^+$ T cells. The [F → M], [II-/-→M] animals (express HY antigen on only non-hematopoietic target cells) died from severe GVHD while the [M → F] animals (express HY antigen on only hematopoietic cells) showed only modest GVHD. Collectively our data (see Table) demonstrate that **(a)** in contrast to the current dogma, epitope spreading is not always required for GVHD **(b)** the immunodominant antigen expression on target tissues is critical and **(c)** this antigen can be efficiently cross-presented by either host or donor APCs.

GVHD across single immuno-dominant Y antigen miHA

Donor T cells (all of B6♀ TCDBM)	Recipient	GVHD mortality * $P < 0.01$
MataHari (CD8 + Tg, 1x106)	B6 (female)	0%
MataHari (CD8 + Tg, 1x106)	B6 (male)	100%
MataHari (CD8 + Tg, 0.1x106)	B6 (male)	33%
Marilyn (CD4 + Tg, 1x106)	B6 (male)	100%
Rachel (CD4 + Tg, 1x106)	B6 (male)	100%
Marilyn (CD4 + Tg, 1x106)	[B6♀ into B6♂	100%
Marilyn (CD4 + Tg, 1x106)	[B6(male) into B6♀	25%
Marilyn (CD4 + Tg, 1x106)	[II-/- into B6♂	100%

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PROSPECTIVE EVALUATION OF PROTEOMIC PATTERN SPECIFIC FOR AGVHD IN MORE THAN 300 PATIENTS

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Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a curative treatment for many hematologic malignancies or hematopoietic dysfunction syndromes in adult patients, but the application is still limited due to major complications, such as severe graft versus host disease (GvHD) and/or infectious complications. Diagnosis of aGvHD is based on clinical features and biopsies, a non invasive, unbiased laboratory test was developed earlier and has been evaluated prospectively and blinded on more than 1290 samples collected from more than 327 patients undergoing allo-HSCT at Hannover Medical School (MHH) and 7 additional clinics. The majority of the patients included was transplanted for hematological malignancies (n = 320), 9 for hematopoietic failure syndromes,